Medical and Pediatric Oncology 28:274–283 (1997)

Renal Function after Conditioning Therapy for Bone Marrow Transplantation in Childhood

Ludwig Patzer, MD,[1,*] Lutz Hempel, MD,[1] Felix Ringelmann,[1]
Joachim Misselwitz, MD,[1] Dietlinde Fuchs, MD,[1] Felix Zintl, MD,[1]
Matthias Brandis, MD,[2] Marc E. deBroe, MD,[3] and Lothar B. Zimmerhackl, MD[2]

The knowledge of renal function in the course of BMT is poor. We prospectively investigated glomerular and tubular function in 42 children who underwent BMT because of malignancy. Seventeen children were transplanted autologously. Investigations were performed before and immediately after the conditioning regimen. Inulin- and creatinine clearance, albuminuria, urine excretion of α_1-microglobulin, β-N-acetylglucosaminidase, alanine-aminopeptidase, intestinal alkaline phosphatase, and Tamm-Horsfall-Protein as well as sodium-and phosphatreabsorption were measured. The patients were classified regarding use of total body irradiation (fTBI) in the conditioning regimen.

Before CR: Glomerular filtration rate (GFR) was not influenced by the underlying diagnosis or previous treatment. Mean GFR was elevated compared with the reference group. Microalbuminuria was elevated in 15% of patients, and mean levels were higher than in the reference group. Proximal tubular dysfunction was indicated by an elevated excretion of α_1-MG in 54%, of β-NAG in 66%, of AAP in 40%,and of IAP in 47%. Fractional sodium excretion was abnormal in 21%, phosphate reabsorption in 5% and THP-excretion in 7% of the patients.

After CR: Creatinine clearance was not affected by CR. After CR α_1-MG, β-NAG, FE_{Na}, AAP, and IAP were increased compared with values before CR. TP/Cl_{cr} was decreased. Excretion of THP was not altered by CR. In patients without fTBI there was a greater increase in α_1-MG excretion and decrease in phosphate reabsorption after CR compared with patients conditioned with fTBI.

We conclude that significant proximal tubular dysfunction is present in about 50–60% of patients before and in nearly all after CR. Distal tubular function was less severely affected. Severity of nephrotoxicity after CR did not correlate with pre-existing abnormalities. **Med. Pediatr. Oncol. 28:274–283.**

Key words: bone marrow transplantation; nephrotoxicity; tubular function; total body irradiation; alpha-1-microglobulin; inulin clearance

INTRODUCTION

Bone marrow transplantation (BMT) is a well established therapy for malignant diseases in childhood, and the list of indications is still growing. The introduction of BMT has increased the rate of cure to 50% in a group of diseases with a previously poor prognosis. This improvement in survival and cure rates has made it both necessary and possible in recent years to study the side effects and complications of therapy. The preparative regimens for BMT have been increasing in intensity to promote marrow engraftment and reduce the incidence of relapse of disease. With this intensification of preparative regimens, as well as with an increase in the number of long-term survivors, new toxicities are becoming apparent [1]. Renal complications have a significant influence on the morbidity and mortality of children with malignant diseases. Clinically relevant complications are the tumour lysis syndrome, which may be associated with acute renal failure in the early phase of antineoplastic therapy, and sepsis-associated renal failure in the phase of marrow aplasia. The nephrotoxicity of several drugs administered

Abbreviations: AAP—alanine-aminopeptidase, ALL—acute lymphoblastic leukaemia, α_1-MG—alpha-1-microglobulin, AML—acute non-lymphoblastic leukaemia, BMT—bone marrow transplantation, β-NAG—β-N-acetylglucosaminidase, Cl_{cr}—creatinine clearance calculated by the formula of Schwartz, Cl_{In}—inulin clearance, CML—chronic myeloid leukaemia, CR—conditioning regimen, FE_{Na}—fractional sodium reabsorption, fTBI—fractionated total body irradiation, GFR—glomerular filtration rate, IAP—human intestinal alkaline phosphatase, RRT—regimen-related toxicity score, THP—Tamm-Horsfall protein, TP/Cl_{cr}—fractional tubular phosphate reabsorption

[1]Children's Hospital, ''Jussuf Ibrahim'', Friedrich Schiller-University, Jena, Germany

[2]Department of Paediatrics, Albert-Ludwigs-University, Freiburg i. Br., Germany

[3]Department of Nephrology-Hypertension, University Hospital, Antwerp, Belgium

Contract grant sponsor: Deutsche Krebshilfe-Mildred Scheel Stifung, contract grant number: M 107/91/Br1; no. 70482.

*Correspondence to: Ludwig Patzer, Friedrich-Schiller-Universität, Klinik für Kinder- und Jugendmedizin, ''Jussuf Ibrahim'' Kochstraβe 2 07743 Jena Germany

Received 29 December 1995; accepted 28 June 1996

in conventional chemotherapy and in BMT has been widely reported [2]. The dose limiting factor of some drugs, used in the supportive therapy of BMT (i.e. cyclosporine A, amphotericin B, and aminoglycosides) is their nephrotoxic potential. Particular attention has been paid in the past five years to tubular toxicity with Fanconi syndrome, which may develop as long as 18 months after ifosfamide therapy [3–8].

Subjects of intensive studies related to BMT were bone marrow nephropathy [9,10], radiation nephritis [11–13], the haemolytic uremic syndrome [14–17] and acute renal failure [18–23]. In contrast our knowledge about tubular renal disorders in connection with BMT is limited. The aim of the present prospective study is to elucidate the glomerular and tubular renal function prior to and immediately after the conditioning regimen preparing for BMT.

PATIENTS AND METHODS

Between September 1992 and January 1995, 42 patients (27 males) were prospectively investigated. The mean age was 12.9 (range 2.7–40.2) years at BMT. Six patients were older than 18 years.

Eighteen patients had acute lymphoblastic leukaemia: 3 in C1 (first remission), 9 in C2, 3 in C3, 2 in R1 (first relapse), and 1 in R2. Twelve had acute nonlymphoblastic leukaemia: 9 in C1, 3 in C2. Five had chronic myeloid leukaemia, 2 Hodgkin's lymphoma, 3 Non Hodgkin's lymphoma, 1 Ewing's sarcoma, and 1 PNET. Seventeen children were transplanted autologously and 25 allogeneically.

No patient had a history of renal disease before diagnosis of malignancy. Renal malformations were excluded by ultrasound. No patient received cis-platinum. The patients in remission had received no cytostatic treatment for at least four weeks before investigation.The patients were investigated about three weeks before BMT. The second investigation was done immediately after the end of CR. The results of further examinations up to two years will be described in a later paper.

All investigations were made strictly in accordance with the guidelines of the local ethical committee and all patients gave informed consent.

Reference Values

As a reference group for the tubular parameters (excretion of α_1-MG, β-NAG, AAP, and THP) 90 healthy children, 15 males and 15 females each in three groups (3 to 6, 6 to 12, and 12 to 18 years; unpublished data, see figures 2–4) were investigated. Reference values of Cl_{In} and Cl_{cr}, calculated by the formula of Schwartz, were measured in 12 healthy volunteers; pathological GFR was defined below 90 ml/min 1.73 m^2. The reference values of FE_{Na}, TP/Cl_{cr}, and IAP were taken from the literature [24,25].

Conditioning regimen

The following CRs were used:

1. Combination of busulfane (BU) and cyclophosphamide (CY) n = 10
   - BU (4 mg/kg for 4 days)/CY (50 mg/kg for 4 days)
2. Combination of BU/CY and etoposide (ETO) n = 10
   - BU (4 mg/kg for 4 days)/CY (2 × 60 mg/kg)/ETO (1 × 40 mg/kg)
3. Combination of fTBI and ETO n = 10
   - fTBI (6 × 2 Gy)/ETO (1 × 60 mg/kg)
4. others with fTBI n = 5
   - fTBI (6 × 2 Gy)/thiotepa (3 × 300 mg/m^2)/ETO (3 × 300 mg/m^2) n = 1
   - fTBI (6 × 2 Gy)/CY (2 × 60 mg/kg)/ETO (1 × 40 mg/kg) n = 3
   - fTBI (6 × 2 Gy)/CY (2 × 60 mg/kg) n = 1
5. others without fTBI n = 7
   - CY (4 × 1,5 g/m^2)/ETO (4 × 250 mg/m^2)/BCNU (1 × 300 mg/m^2)/AraC (4 × 200 mg/m^2) n = 2
   - BU (4 × 4 mg/kg)/ETO (1 × 40 mg/kg) n = 1
   - BU (4 × 30 mg/kg)/ETO (3 × 300 mg/m^2)/thiotepa (3 × 300 mg/m^2) n = 2
   - ETO (1 × 50 mg/kg)/melphalan (4 × 30 mg/m^2)/carboplatin (3 × 500 mg/m^2) n = 2

Study Design and Laboratory Investigations

Urine was collected for about 17 hours overnight and stored at 4°C. The activity of urinary enzymes was measured within 7 days. Urine samples for determination of α_1-MG, THP, and albumin were frozen at −18°C until measurement.

The following morning a fasting blood sample was taken and processed immediately and the inulin clearance was performed.

The following variables were investigated:

Glomerular Function

- single shot injection inulin clearance done by the method of Gretz [26]. Because of the reversed isolation of the patients, the Cl_{In} was done before BMT and later than 30 days after BMT
- creatinine clearance, calculated by the formula of Schwartz [27,28]
- albumin (nephelometric assay, ARRAY 360 Beckmann Co.)

Tubular Function

- α_1-MG: commercially available solid phase ELISA (Elias Co. Freiburg F.R.G.)
- β-N-acetylglucosaminidase: commercially available colorimetric assay (Boehringer Co. Mannheim F.R.G.) alanine-aminopeptidase [29]
- human intestinal alkaline phosphatase: ELISA [25]

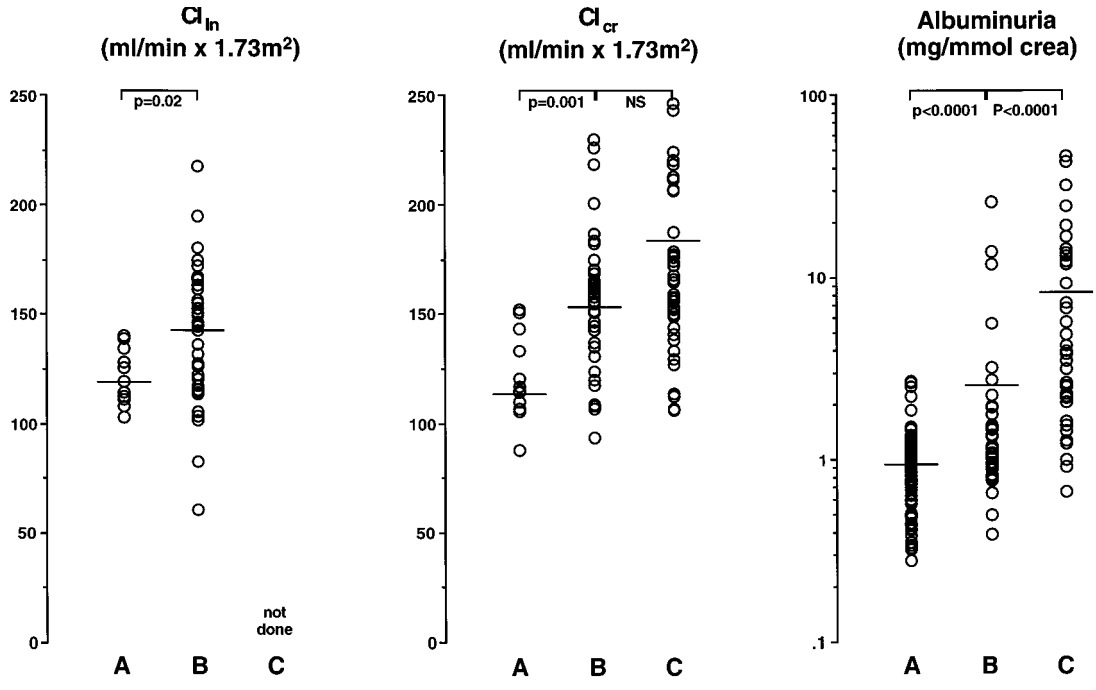

Fig. 1. Glomerular renal function. Comparison between the reference group (A) and the parameters of the patients before (B) and after CR (C). The horizontal lines indicate the means.

- fractional excretion of sodium: $FE_{Na}[\%] = (U_{Na} \times P_{cr}/P_{Na} \times U_{cr}) \times 100$
- fractional tubular phosphate reabsorption: TP/Cl_{cr} $[mmol/l] = P_{PO4} - (U_{PO4} \times P_{cr})/U_{cr}$
- Tamm-Horsfall-Protein: commercially available solid phase ELISA (Elias Co. Freiburg F.R.G.)

Serum

The concentration of sodium and potassium were measured by ionsensitive electrodes. Calcium, phosphate, creatinine, and urea were measured on a CX3/CX4 colorimetrically (Beckmann Co.).

Statistics

A pathological value was defined as one below the 3rd or above the 97th percentile of the reference values. The statistical procedures were performed using SPSS for Windows® V 6.02. For comparison of percentages of pathological values before and after CR McNemar's Chi square test for matched samples was used as described in [42]. The statistical difference between values of two independent groups was done with the Mann-Whitney U-Wilcoxon Rank Sum W test. To compare more than 2 independent groups we used the Kruskal-Wallis 1-Way Anova. The Wilcoxon Matched-Pairs Signed-Ranks test was used to compare the results before and after CR. The multiple linear regression method (stepwise forward procedure) was used to prove the dependence of the measured variables from some independent features of the patients.

RESULTS

Glomerular Function before CR

Glomerular filtration rate. GFR was slightly but significantly higher in the patients compared with controls (fig. 1). GFR was lower than the normal range in only 2 patients before CR (fig. 2). There was no significant difference between Cl_{ln} and Cl_{cr} (Wilcoxon matched pairs test).

There were no significant differences in GFR between the groups in respect to the underlying diagnosis or remission status in ALL and AML (table 1.)

Albuminuria. Microalbuminuria was greater than normal in 15% of patients (fig. 2). Patient levels were significantly elevated in comparison to the reference group (fig. 1). There were no statistical significant differences in respect to the underlying diagnosis, remission status, cumulative ifosfamide, or MTX dose. The degree of albuminuria did not correlate with GFR.

Tubular Function before CR

Figure 2 shows the percentage of pathological findings in tubular function before CR. An increased excretion of α_1-MG was seen in 54%, of β-NAG in 66%, of AAP in 40% and of IAP in 47% of the patients. The fractional excretion of sodium was elevated in 21% and the reabsorption of phosphate was decreased in 5% of all patients.

The excretion of THP was reduced in 7% of all patients. The excretion of α_1-MG, β-NAG and AAP was

TABLE I. Glomerular Renal Function Depending on the Underlying Diagnosis and Remission Status before CR

	ALL						AML			CML	Solid Tumours	*P*
	All	C1	C2	C3	R1	R2	All	C1	C2			
Cl_{in} [ml/min × 1.73 m^2]	148 ± 30	182 ± 31	133 ± 26	144 ± 22	134 ± 26	172	135 ± 31	125 ± 32	159 ± 11	142 ± 13	133 ± 43	NS
	103 – 217	155 – 217	103 – 180	122 – 167	115 – 152		83 – 172	83 – 172	146 – 167	127 – 161	60 – 194	
Cl_{Cr} [ml/min × 1.73m^2]	153 ± 28	188 ± 28	150 ± 25	142 ± 31	136 ± 1	170	150 ± 42	143 ± 34	167 ± 61	171 ± 22	161 ± 40	NS
	106 – 218	163 – 218	108 – 183	106 – 163	135 – 137		106 – 229	108 – 192	106 – 229	146 – 200	93 – 226	
Albuminuria [mg/mmol crea]	1.6 ± 1.2	2.1 ± 0.6	1.1 ± 0.4	2.5 ± 2.7	1.4 ± 0.02	0.8	3.9 ± 7.8	4.8 ± 9.4	1.9 ± 1.2	3.6 ± 5.8	2.8 ± 4.0	NS
	0.4 – 5.6	1.5 – 2.8	0.4 – 1.5	0.9 – 5.6	1.34 – 1.37		0.8 – 26.0	0.8 – 26.0	0.8 – 3.2	0.5 – 13.9	0.9 – 11.9	
Patients n =	16	3	8	3	2	1	10	7	3	5	7	

The *p*-values indicate the statistical significance between the four groups (ALL, AML, CML, and solid tumours). (Kruskal-Wallis 1-Way Anova)
Shown is the mean ± standard deviation and the range. (NS - not significant)

significantly higher in patients than in controls (fig. 3). Also the excretion of FE_{Na} and IAP was elevated and TP/CL_{cr} was markedly decreased, compared with published values [24,25] (fig. 4).The excretion of THP (indicating distal tubule function) was the same as in our reference group (fig. 4).

Tubular function before CR did not correlate with the underlying diagnosis or with remission status in patients with ALL and AML (Table II), (although the excretion of α_1-MG-and β-NAG-excretion was slightly but not significantly higher in patients with CML and solid tumours than in patients with ALL and AML.)

Glomerular Function after Conditioning Regimen

Glomerular filtration rate. There was a slight but not significant increase in Cl_{cr} after CR (fig. 1); no patient had a reduction in GFR after CR. Thus GFR was not affected immediately after CR (fig. 2). A significantly lower, but still normal, Cl_{cr} was found in patients conditioned without fTBI, compared with those who received fTBI (Table III).

Albuminuria. In contrast to Cl_{cr}, albuminuria was significantly higher after CR than before (fig. 1). In 54% of patients pathological albuminuria was detectable immediately after CR (fig. 2). Patients conditioned without fTBI had significantly higher albuminuria than patients conditioned with fTBI, consistent with results of Cl_{cr} (Table III). Prior to CR albuminuria did not differ between these groups.

Also the difference in albuminuria before and after CR was significantly higher in patients without fTBI, i.e. albuminuria rises more in patients conditioned without fTBI. Albuminuria after CR did not correlate with the cumulative doses of ifosfamide and methotrexate or with albuminuria before CR, using a linear multiple regression model.

Tubular Function after Conditioning

The frequencies of pathological findings investigating the renal tubular function are given in figure 2.

An elevated excretion of α_1-MG was found in 95%, of β-NAG in 98%, of AAP in 75%, and of IAP in 82%. FE_{Na} was elevated in 49%, TP/Cl_{cr} low only in 10%, and THP low in 10%. The change in percentage of pathological value was not significant in TP/Cl_{cr} and THP. Compared with results before CR α_1-MG-, β-NAG-, AAP-, IAP-excretion as well as FE_{Na} were significantly increased and TP/Cl_{cr} significantly decreased (see fig. 3 and 4). In contrast the excretion of THP was not significantly altered by CR.

Patients without fTBI had significantly higher excretion of α_1-MG and lower TP/CL_{cr} (Table III) compared with patients conditioned with fTBI. These tubular parameters did not differ between the groups before CR.

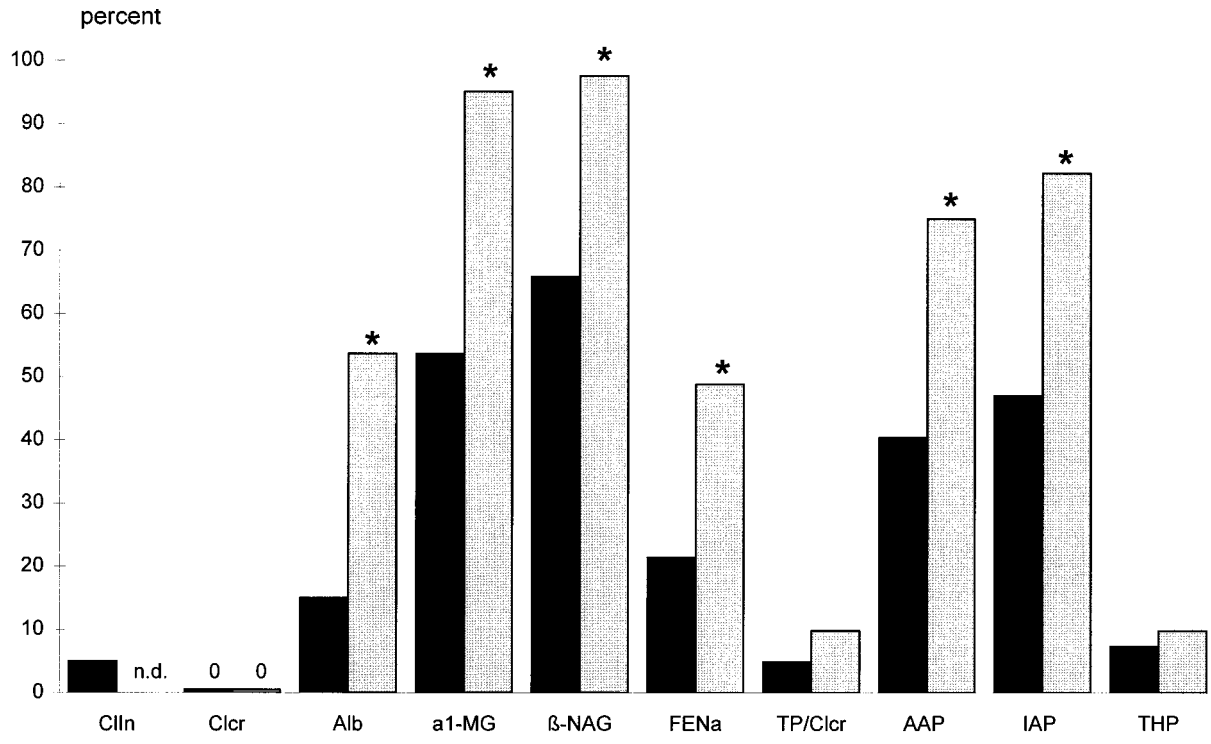

Fig. 2. Percentage of pathological findings of glomerular (Cl_{in}, Cl_{cr}, Alb) and tubular parameters (α_1-MG, β-NAG, FE_{Na}, TP/Cl_{cr}, AAP, IAP and THP) before (black columns) and after CR (grey columns). Asterixes indicate significant difference compared with before CR at $p < 0.05$ (n.d. - not done)

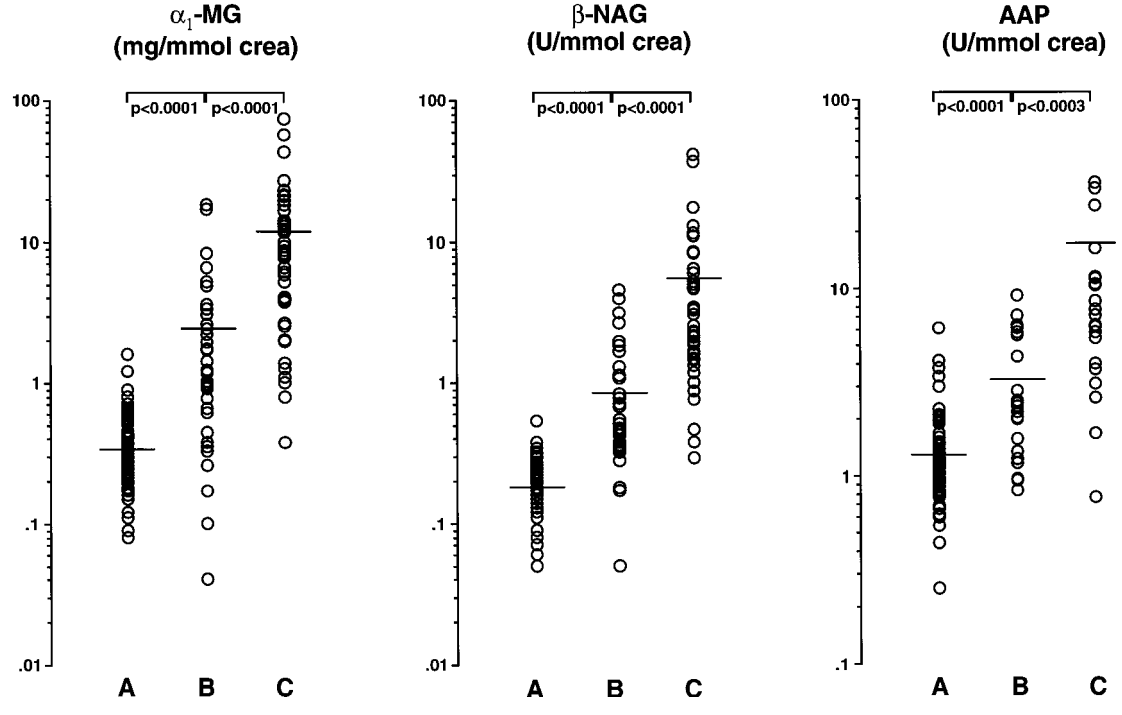

Fig. 3. Tubular renal function. Comparison between the reference group (A) and the parameters of the patients before (B) and after CR (C). The horizontal lines indicate the means.

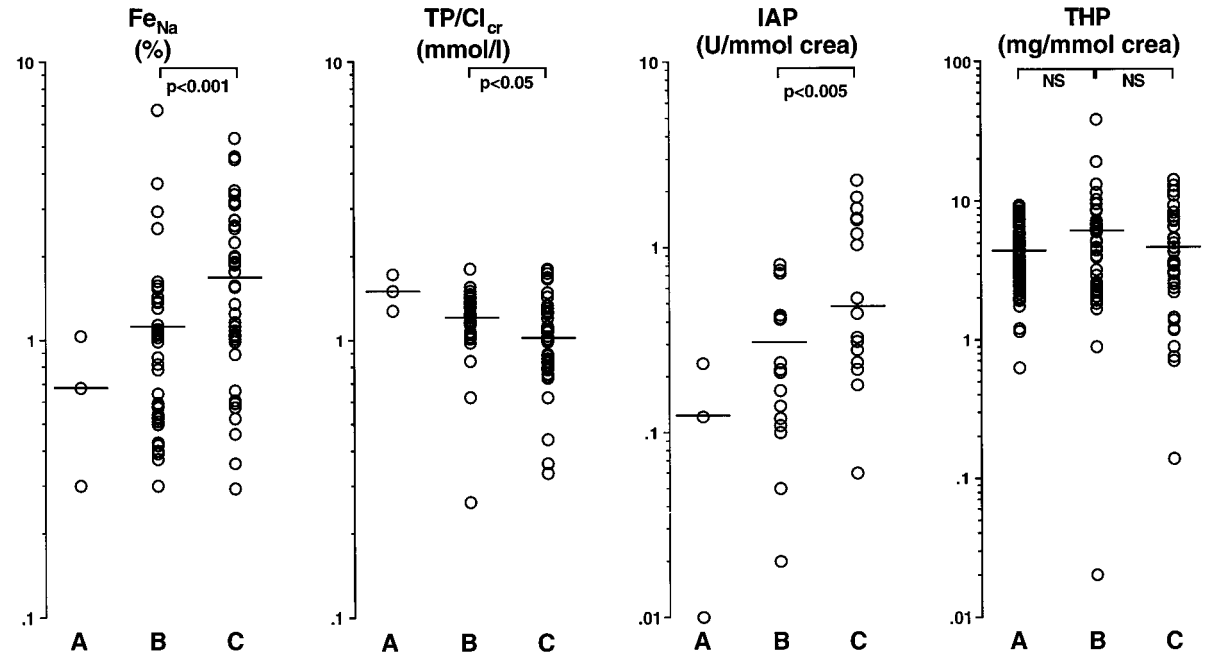

Fig. 4. Tubular renal function. Comparison between the reference group (A) and the parameters of the patients before (B) and after CR (C). The horizontal lines indicate the means. The reference values (mean ± standard deviation) of FENa and TP/Clcr were taken from [24]. The reference values of IAP (5th, 50th and 95th percentiles) were taken from [25]. NS = not significant.

TABLE II. Tubular Renal Function Depending on the Underlying Diagnosis Before CR. The *p*-values Indicate the Statistical Significance Between the Four Groups (ALL, AML, CML, and Solid Tumours). Kruskal-Wallis 1-way Anova. Shown is the Mean ± Standard Deviation and the Range

	ALL	AML	CML	Solid tumours	
α_1-MG	1.75 ± 1.97	1.44 ± 2.06	2.13 ± 1.82	6.21 ± 7.84	$p = 0.151$
[mg/mmol crea]	0.17 – 8.35	0.04 – 6.6	0.62 – 5.16	0.35 – 18.1	
β-NAG	0.68 ± 0.72	0.84 ± 1.19	1.29 ± 1.54	1.23 ± 0.92	$p = 0.539$
[U/mmol crea]	0.18 – 3.1	0.05 – 4.5	0.17 – 3.94	0.28 – 2.67	
FE_{Na}	0.83 ± 0.46	1.45 ± 1.76	1.20 ± 1.08	1.31 ± 1.09	$p = 0.744$
[%]	0.3 – 1.57	0.30 – 6.72	0.42 – 2.92	0.42 – 2.92	
TP/Cl_{cr}	1.29 ± 0.26	1.24 ± 0.35	1.02 ± 0.27	1.22 ± 1.27	$p = 0.145$
[mmol/l]	0.84 – 1.82	0.26 – 1.51	0.62 – 1.38	1.06 – 1.42[c]	
AAP	2.83 ± 2.12	4.26 ± 3.93	3.18 ± 2.60	3.15 ± 1.90	$p = 0.968$
[U/mmol crea]	0.84 – 7.12[e]	0.95 – 9.1[b]	1.34 – 6.16[a]	0.97 – 6.31[c]	
IAP	0.23 ± 0.23	0.33 ± 0.42	0.43 ± 0.30	0.35 ± 0.12	$p = 0.624$
[U/mmol crea]	0.05 – 0.76[d]	0.02 – 0.8[a]	0.12 – 0.72[a]	0.21 – 0.42	
THP	5.84 ± 3.43	6.64 ± 10.36	5.64 ± 7.7	6.17 ± 2.9	$p = 0.382$
[mg/mmol crea]	0.89 – 13.2	0.02 – 38.6	1.66 – 19.4	2.33 – 9.46	
Patients					
n =	17	12	5	7	

([a] - results were available in 3 patients, [b] - results were available in 4 patients, [c] - results were available in 6 patients, [d] - results were available in 8 patients, [e] - results were available in 9 patients)

DISCUSSION

Renal functional impairment after BMT has been quite extensively studied [9, 11, 13, 14, 16, 18, 20, 22, 30–33]. Cyclosporine A associated renal injury, radiation nephritis, and a haemolytic uremic like syndrome are the most important clinical problems. However little is known about the impact of CR on renal function, especially on tubular function.The purpose of the present study was to prospectively evaluate tubular and glomerular function in the course of BMT, in a representative group of children.

The first step was to evaluate renal function before BMT. GFR was decreased only in two patients. This is lower than reported by van Why et al., who found elevated serum creatinine in 8 of 64 patients before BMT, in a retrospective study [33]. All of a group of 14 patients with late renal dysfunction after BMT reported by Guinan et al. had normal creatinine clearance before BMT

TABLE III. Glomerular and Tubular Function after CR Depending on the Use of fTBI in the Conditioning Regimen. The *p*-values Indicate the Statistical Significance of the Difference Between Both Groups. Shown is the Mean ± Standard Deviation and the Range. Mann-Whitney U-Wilcoxon Rank Sum W Test

	fTBI	No fTBI	
Cl_{Cr}	189 ± 35	160 ± 37	$p = 0.019$
[ml/min × 1.73m^2]	129 – 246	106 – 253	
Albuminuria	4.0 ± 6.2	10.7 ± 12.2	$p = 0.012$
[mg/mmol crea]	0.7 – 24.6	0.9 – 46.3	
α_1-MG	5.2 ± 5.3	16.6 ± 17.4	$p = 0.0042$
[mg/mmol crea]'	0.8 – 19.6	0.38 – 74.7	
βNAG	2.98 ± 2.92	7.02 ± 10.14	$p = 0.169$
[U/mmol crea]	0.29 – 10.96	0.38 – 41.6	
FE_{Na}	1.66 ± 1.3	1.9 ± 1.3	$p = 0.518$
[%]	0.29 – 4.57	0.46 – 5.35	
TP/Cl_{cr}	1.23 ± 0.37	0.99 ± 0.38	$p = 0.025$
[mmol/l]	0.44 – 1.78	0.33 – 1.8	
AAP	8.5 ± 4.5[a]	12.2 ± 12.3[b]	$p = 0.944$
[U/mmol crea]	3.1 – 16.3	0.77 – 36.4	
IAP	0.88 ± 0.8[a]	5.33 ± 16.1[c]	$p = 0.88$
[U/mmol crea]	0.22 – 1.86	0.06 – 58.8	
THP	6.29 ± 4.03	4.11 ± 3.31	$p = 0.093$
[mg/mmol crea]	0.14 – 12.8	0.7 – 14.1	
Patients n =	14	27	

([a] - results were available in 6 patients, [b] - results were available in 17 patients, [c] - results were available in 13 patients).

[11]. We could not detect a difference in the GFR depending on the underlying diagnosis or on the remission status. This is in contrast to the report of Berg et al. [31] who found a decreased GFR prior to BMT in patients with ALL compared with that in controls and patients with AML. This was attributed to the higher dose of cytostatic drugs in patients with ALL, who were usually transplanted in a later remission than those with AML.

Surprising findings were the significant elevated Cl_{ln} and Cl_{cr} before CR. All patients were in a stable nutritional state, without parenteral nutrition or fluid and without steroids. The elevated GFR may be caused by cytostatic treatment and/or the supporting therapy; however the mechanism is not clear. Elevated GFR before BMT has not previously been described. A more sensitive parameter indicating glomerular injury seems to be the excretion of albumin. Prior to BMT we found an increased value in 15% of all patients. The elevation in albumin excretion may be caused by a toxic injury of the basement membrane or by hyperfiltration. We were not able to define a risk factor for albuminuria.

We found a high incidence of tubular dysfunction in our patients: a group with poor prognosis of their malignant disease, in different states of remission, and who had received a range of different intensive cytostatic treatment. We found an injury of the proximal tubule in about 50–60% depending on the investigated variable and a disturbance of the distal tubule only in 7%. In comparison with our own reference values α_1-MG, β-NAG, and AAP-excretion were significantly elevated in the patients. In comparison with normal values from the literature FE_{Na} and IAP-excretion were also elevated, and TP/CL_{cr} was decreased. Tubular injury in our patients did not seem to be related to the previous use of ifosfamide or other nephrotoxic agents. In contrast, Ashraf [3], Rossi [5], and Skinner and Jones [7,8] reported nephrotoxicity after ifosfamide treatment.

One reason could be the relatively low dose of ifosfamide in the patients with haematological malignancies (4 patients got 6 g/m^2, 1 got 12, and one 30 g/m^2 ifosfamide). One of our patients who received 84 g/m^2 ifosfamide showed a severe functional impairment in all glomerular and tubular parameters except THP.

To define the effect on renal function of CR alone, without the influence of other factors such as bone marrow infusion, CyA-toxicity, GvHD, sepsis or nephrotoxic drugs, we have investigated the children immediately after CR, but before BMT. No patient showed a doubling of serum creatinine immediately after CR, but during follow up 11 of 42 patients had a doubling of serum creatinine within 30 days after BMT.

There was pathological albuminuria in 54% of the patients. The mean value was elevated 8.8 fold compared with the reference value, and 3.2 fold compared with the results before CR, indicating that CR caused significant injury to the glomerular basement membrane. There are no published reports on the impact of any type of CR on renal function. Our findings must therefore be compared with reports of findings after BMT. Nevill et al. [34] reported a low incidence of renal toxicity using a ''regimen-related toxicity score'' (RRT) after CR with busulfane and cyclophosphamide, in a retrospective study of 70 patients. This score, described by Bearman et al. [30], defines renal toxicity depending only on GFR (grade I = increase in creatinine up to twice the baseline value before the start of conditioning, grade II = increase in creatinine above twice baseline but not requiring dialysis, grade III = requirement of dialysis, grade IV = fatal toxicity). In the report from Nevill et al. the times of investigation and of manifestation of organ toxicity are not given. No toxicity was seen in 65% of patients, grade I in 26% and grade II in 9%. Grade III and IV toxicity were not observed. The same toxicity score was used by Cowen et al. [32], who reported 100 patients undergoing CR with fTBI and cyclophosphamide 60 mg/kg/day for 2 days. All 22 patients with autologous transplants had no renal toxicity.

Cole et al. reported 21 children undergoing CR with fTBI, etoposide and cyclophosphamide using the same toxicity score [35]. One patient died 3 months after BMT as a result of haemolytic uremic syndrome (HUS) and one patient had mild HUS that resolved spontaneously. There was no renal toxicity in 11 patients, grade I in 6 and grade II in 3. Przepiorka et al. reported renal toxicity in thirty adults after a CR with different doses of high-dose thiotepa, busulfane, and cyclophosphamide, using

the same score [36]. Three patients had grade I or II toxicity and one patient grade III-IV. In another report of 127 patients from the same group using the same CR, 6 patients had grade I or II and 3 grade III or IV of renal toxicity [37]. Rosenthal et al. have reported the RRT-score associated with busulfane and cyclophosphamide in autologous BMT [38]. They reported renal toxicity grade I in 5% and also grade II in 5% in 69 patients retrospectively reviewed and concluded that this CR was associated with minimal non-haemopoietic toxicity.

Fields et al. reported that the dose-limiting toxicities of intensive doses of ifosfamide, carboplatin, and etoposide followed by autologous stem cell rescue included acute renal failure [39], and that ''significant renal wasting of potassium, magnesium, calcium, phosphorous, and bicarbonate was noted'' [40].

Gordon et al. [41] reported ''unexpectedly severe renal failure'' in patients conditioned with vincristine, melphalan, etoposide, and carboplatin in advanced neuroblastoma. The authors measured the GFR by the ^{51}Cr-EDTA-clearance before chemotherapy and at day +7. Four of the 16 patients developed acute renal failure and 15 showed a rise in serum creatinine. The authors concluded that in these patients the predominant and most serious non-haematologic toxicity was renal.

Zager reviewed acute renal failure as one of the most frequent and potentially life threatening complications of BMT [22]. Approximately 40% of patients develop renal insufficiency early in the course of BMT and about 50% of them require dialysis. No reports exist dealing with extensive investigation of tubular function in the course of BMT.

In our study a very high percentage of children had tubular dysfunction. There was elevation of α_1-MG (a marker of proximal tubular structural injury) in 95% and elevation of IAP (a marker of injury to the S3-segment) in 82%. In contrast decreased THP-secretion (a marker of distal tubular damage) was seen in only 10%. CR led to significant alteration in all measured tubular variables except THP, indicating severe toxicity to the proximal tubule. In contrast, damage to the distal tubule is relatively mild. The tubular function after CR did not correlate with pre-existing damage.We compared patients conditioned with and without fTBI to examine the influence of fTBI on renal function. Prior to CR measured variables did not differ significantly between the groups. After CR children conditioned without fTBI had significant lower GFR (but still in the normal range), significant higher albuminuria and α_1-MG-excretion, and significantly lower phosphate reabsorption. There was a significant correlation between the use of fTBI and the creatinine clearance and α_1-MG excretion after CR (linear multiple regression). These findings suggest that CR with cytostatic drugs (without fTBI) leads to more tubular toxicity early after conditioning. The contribution of severely disturbed tubular function to the mortality and morbidity in critically ill children, especially in children immediately after BMT is not known. Although enhanced excretions of α_1-MG and β-NAG themselves do not cause any unfavourable effect on the patient they are highly indicative for other proximal tubular dysfunctions as leakage of bicarbonate and electrolytes. The clinical management of those patients with disturbed proximal tubular function has to take into account that these children are not able to regulate their plasma biochemistry to some extent. So acid balance, electrolyte, and water homeostasis should be assessed very carefully.

There are no published studies suggesting beneficial effect of any measures on tubular function in children treated with cytotoxic drugs. In case of antibiotic treatment, aminoglycosides and amphotericin B should be avoided if possible. From our point of view children undergoing CR for BMT should be treated according to the established guidelines of cytotoxic treatment with vigorous hydration and urinary urine alkalinization.

From our study no recommendations can be given to improve tubular changes in these patients.

CONCLUSION

These results, from 42 patients with different malignant diseases, different cytostatic treatments, different conditioning regimes, and different types of BMT, suggest an important effect on renal function of myeloablative cytostatic treatment. Children admitted for BMT had relatively normal glomerular function but 50–60% had tubular dysfunction. This would not be detected by the usual organ toxicity score or routine renal testing (serum creatinine, urea, urine microscopy). We would like to emphasise that the often used ''regimen-related toxicity score'' is a very rough estimate of kidney function. After CR prior to BMT nearly all children suffer from proximal tubular toxicity, but there are no markers which can be used to predict the later course of renal function. Preliminary results of a two-year follow-up of these children show long lasting minimal tubular dysfunction with a variable degree of recovery, and will be detailed in another report.

ACKNOWLEDGMENTS

The authors thank Mrs. Isolde Bernhardt, Mrs. Angelika Brecht, Mrs. Monika Mack-Vetter, and Mrs. Monika Kramer for excellent technical assistance and data management and the nursing staff of BMT-unit for providing excellent care of the patients.

This work was supported by grant of ''Deutsche Krebshilfe-Mildred Scheel Stiftung'' M 107/91/Br 1; no. 70482; we thank for their financial support.

REFERENCES

1. Tarbell NJ, GuinanEC, NiemeyerC, Mauch P, Sallan SE, Weinstein HJ: Late onset of renal dysfunction in survivors of bone marrow transplantation. Int J Radiat Oncol Biol Phys 15:99–104, 1988.
2. Brandis M, von der Hardt K, Zimmerhackl RB, Mohrmann M, Leititis J: Cytostatics-induced tubular toxicity. Clin Invest 71: 855–857, 1993.
3. Ashraf MS, Brady J, Breatnach F, Deasy PF, O'meara A: Ifosfamide nephrotoxicity in paediatric cancer patients. Eur J Pediatr 153:90–94, 1994.
4. Potter R, Roes F, Schellong G, Bartenstein P, Bramswig JH, von Lengerke HJ, Rath B, Mohring R, Rossi R: Subclinical impairment of renal function after radiotherapy for Hodgkin's disease in children. Recent Results Cancer Res 130:259–267, 1993.
5. Rossi R, Ehrich JH: Partial and complete de Toni-Debre-Fanconi syndrome after ifosfamide chemotherapy of childhood malignancy. Eur J Clin Pharmacol 44 Suppl 1:S43–5, 1993.
6. Rossi R, Kleinebrand A, Godde A, Rath B, Jurgens H: Increased risk of ifosfamide-induced renal Fanconi's syndrome after unilateral nephrectomy [letter]. Lancet 341:755, 1993
7. Jones DP, Chesney RW: Renal toxicity of cancer chemotherapeutic agents in children: ifosfamide and cisplatin. Curr. Opin. Pediatr. 7 (2):208–213, 1995.
8. Skinner R, Pearson AD, Price L, Coulthard MG, Craft AW: Nephrotoxicity after ifosfamide. Arch Dis Child 65:732–738, 1990.
9. Cohen EP, Lawten CA, Moulder JE, Becker CG, Ash RC: Clinical course of late-onset bone marrow transplant nephropathy. Nephron 64:626–635, 1993.
10. Cohen EP, Moulder JE, Fish BL, Hill P: Prophylaxis of experimental bone marrow transplant nephropathy [see comments]. J Lab Clin Med 124:371–380, 1994.
11. Guinan EC, Tarbell NJ, Niemeyer CM, Sallan SE, Weinstein HJ: Intravascular hemolysis and renal insufficiency after bone marrow transplantation. Blood 72:451–455, 1988.
12. Moulder JE, Fish BL: Late toxicity of total body irradiation with bone marrow transplantation in a rat model. Int J Radiat Oncol Biol Phys 16:1501–1509, 1989.
13. Tarbell NJ, Guinan EC, Chin L, Mauch P, Weinstein HJ: Renal insufficiency after total body irradiation for pediatric bone marrow transplantation. Radiother Oncol 18 Suppl 1:139–142, 1990.
14. Carlson K, Smedmyr B, Hagberg H, Oberg G, Simonsson B: Haemolytic uraemic syndrome and renal dysfunction following BEAC (BCNU, etoposide, ara-C, cyclophosphamide) +/− TBI and autologous BMT for malignant lymphomas. Bone Marrow Transplant 11:205–208, 1993.
15. Down JD, Berman AJ, Warhol M, Yeap B, Mauch P: Late complications following total-body irradiation and bone marrow rescue in mice: predominance of glomerular nephropathy and hemolytic anemia. Int J Radiat Biol 57:551–565, 1990.
16. Juckett M, Perry EH, Daniels BS, Weisdorf DJ: Hemolytic uremic syndrome following bone marrow transplantation. Bone Marrow Transplant 7:405–409, 1991.
17. Oursler DP, Holley KE, Wagoner RD: Hemolytic uremic syndrome after bone marrow transplantation without total body irradiation. Am J Nephrol 13:167–170, 1993.
18. Attal M, Huguet F, Rubie H, Charlet JP, Schlaifer D, Huynh A, Laurent G, Pris J: Prevention of regimen-related toxicities after bone marrow transplantation by pentoxifylline: a prospective, randomized trial. Blood 82:732–736, 1993.
19. Elias AD, Ayash LJ, Eder JP, Wheeler C, Deary J, Weissman L, Schryber S, Hunt M, Critchlow J, Schnipper L,: A phase I study of high-dose ifosfamide and escalating doses of carboplatin with autologous bone marrow support. J Clin Oncol 9:320–327, 1991.
20. Lane PH, Mauer SM, Blazar BR, Ramsay NK, Kashtan CE: Outcome of dialysis for acute renal failure in pediatric bone marrow transplant patients. Bone Marrow Transplant 13:613–617, 1994.
21. Lönnerholm G, Carlson K, Bratteby L-E, Bäcklund L, Hagberg H, Rikner G, Smedmyr B, Oberg G, Simonsson B: Renal function after autologous bone marrow transplantation. Bone Marrow Transplant 8:129–134, 1991.
22. Zager RA: Acute renal failure in the setting of bone marrow transplantation. Kidney Int 46:1443–1458, 1994.
23. Zager RA, O'Quigley J, Zager BK, Alpers CE, Shulman HM, Gamelin LM, Stewart P, Thomas ED: Acute renal failure following bone marrow transplantation: a retrospective study of 272 patients. Am J Kidney Dis 13:210–216, 1989.
24. Rossi R, Danzebrink S, Linnenbürger K, Hillebrand D, Grüneberg M, Sablitzky V, Deufel T, Ullrich K, Harms E: Assessment of tubular reabsorption of sodium, glucose, phosphate and amino acids based on spot urine samples. Acta Paediatr 83:1282–1286, 1994.
25. Verpooten GF, Nuyts GD, Hoylaerts MF, Nouwen EJ, Vassanyiova Z, Dhlopolchek P, deBroe ME: Immunoassay in urine of a specific marker for proximal tubular S3-segment. Clin Chem 38: 642–647, 1992.
26. Gretz N, Ecker W, Tschirner KH, Kuhnle HF, von Dahl K, Kirschfink M, Drescher P, Lasserre JJ, Strauch M: Practicability of the inulin plasma single-shot clearance. Contrib Nephrol 81:220–228, 1990.
27. Schwartz GJ, Gauthier B: A simple estimate of glomerular filtration rate in adolescent boys. J Pediatr 849–854, 1984.
28. Schwartz GJ, Haycock GB, Edelmann CM, Spitzer A: A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. J Pediatr 259–263, 1976.
29. Jung K, Scholz D: An optimized assay alanine aminopeptidase activity in urine. Clin Chem 26:1251–1254, 1980.
30. Bearman SI, Appelbaum FR, Buckner CD, Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol 6:1562–1568, 1988.
31. Berg U, Bolme P: Renal function in children following bone marrow transplantation. Transplant Proc 21:3092–3094, 1989.
32. Cowen D, Richaud P, Marit G, Conymakoul P, Trouette R, Faberes C, Reiffers J: Regimen-related toxicity in patients undergoing BMT with total body irradiation using a sweeping beam technique. Bone Marrow Transplant 10:515–519, 1992.
33. Van Why SK, Friedman AL, Wei LJ, Hong R: Renal insufficiency after bone marrow transplantation in children. Bone Marrow Transplant 7:383–388, 1991.
34. Nevill TJ, Barnett MJ, Klingemann HG, Reece DE, Shepherd JD, Phillips GL: Regimen-related toxicity of a busulfane-cyclophosphamide conditioning regimen in 70 patients undergoing allogeneic bone marrow transplantation. J Clin Oncol 9:1224–1232, 1991.
35. Cole CH, Pritchard S, Rogers PC, Davis JE, Phillips G, Chan KW: Intensive conditioning regimen for bone marrow transplantation in children with high-risk haematological malignancies. Med Pediatr Oncol 23:464–469, 1994.
36. Przepiorka D, Ippoliti C, Giralt S, van Beisen K, Mehra R, Deisseroth AB, Andersson B, Luna M, Cork A, Lee M: A phase I-II study of high-dose thiotepa, busulfane and cyclophosphamide as a preparative regimen for allogeneic marrow transplantation. Bone Marrow Transplant 14:449–453, 1994.
37. Przepiorka D, Dimopoulos M, Smith T, Ippoliti C, Diener K, Luna M, Champlin RE: Thiotepa, busulfane, and cyclophosphamide as a preparative regimen for marrow transplantation: risk factors for early regimen-related toxicity. Ann Hematol 68:183–188, 1994.
38. Rosenthal MA, Grigg AP, Sheridan WP: High dose busulphan/

cyclophosphamide for autologous bone marrow transplantation is associated with minimal non-hemopoietic toxicity. Leuk Lymphoma 14:279–283, 1994.
39. Fields KK, Elfenbein GJ, Perkins JB, Janssen WE, Ballester OF, Hiemenz JW, Zorsky PE, Kronish LE, Foody MC: High-dose ifosfamide/carboplatin/etoposide: maximum tolerable doses, toxicities, and hematopoietic recovery after autologous stem cell reinfusion. Semin Oncol 21:86–92, 1994.
40. Fields KK, Perkins JP, Hiemenz JW, Zorsky PE, Janssen WE, Kronish LE, Machak MC, Elfenbein GJ: Intensive dose ifosfamide, carboplatin, and etoposide followed by autologous stem cell rescue: results of a phase I/II study in breast cancer patients. Surg Oncol 2:87–95, 1993.
41. Gordon SJ, Pearson AD, Reid MM, Craft AW: Toxicity of single-day high-dose vincristine, melphalan, etoposide and carboplatin consolidation with autologous bone marrow rescue in advanced neuroblastoma [see comments]. Eur J Cancer 28A:1319–1323, 1992.
42. Strike P.W. ‘‘Statistical methods in laboratory medicine’’ Oxford: Butterworth-Heinemann, 1991, pp 495–498.